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THE CONTROL OF EGG MATURATION BY JUVENILE HORMONE IN THE TOBACCO HORNWORM MOTH, *MANDUCA SEXTA*

MARY M. NIJHOUT [1] AND LYNN M. RIDDIFORD [1]

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

In a variety of insect species, the adult female requires the presence of active corpora allata (CA) for normal ovarian development. The CA, at this stage, secrete juvenile hormone (JH) which is, presumably, the same compound(s) responsible for the maintenance of larval characters during the early development of the individual (Wigglesworth, 1948; Williams, 1961; deWilde and deLoof, 1973). In *Manduca sexta,* Judy, Schooley, Dunham, Hall, Bergot, and Siddall (1973) have found at least one JH which is secreted by the adult female CA *in vitro* and which can be extracted from larval blood. In some species, JH appears to control the synthesis of the female specific yolk protein (see Engelmann, 1970; Wigglesworth, 1970; Wyatt, 1972; Doane, 1973; deWilde and deLoof, 1973 for reviews). But this is by no means a general rule. Those species which have been shown to have a vitellogenin protein not controlled by JH belong to the Saturniidae (Pan and Wyatt, 1971; Telfer, 1965) and the Culicidae (Hagedorn and Fallon, 1973).

The action of JH in ovarian development in adult female insects is not limited to control of yolk protein synthesis. In some cockroaches, oocytes will not incorporate vitellogenin unless JH is present (Bell, 1969; Bell and Barth, 1971). In *Aedes aegypti,* although synthesis of vitellogenin is not controlled by JH, the CA are required for egg maturation (Lea, 1963, 1967). In this instance, JH acts to promote development of very young follicles to the normal resting stage (Gwadz and Spielman, 1973).

Sroka and Gilbert (1971) first reported that JH was necessary for egg development in the adult female sphinx moth, *Manduca sexta.* Our study was concerned with the role played by JH in this ovarian maturation. The evidence points to a new type of gonadotropic action of JH that is not concerned with either vitellogenin synthesis nor its uptake. Rather JH seems to be permissive in allowing oocytes to mature beyond the vitellogenic phase.

Materials and Methods

Experimental animals

The rearing procedure for larval tobacco hornworms has been described (Truman, 1972). Larvae were raised at 25° C in a long day photoperiod (17L:7D). Developing adults were stored in polyethylene bags and were switched to a short day photoperiod (12L:12D) at 25° C, two weeks after pupation. Adult eclosion is more synchronous under the 12L:12D photoperiod (J. W. Truman, M. M. Nijhout, personal observations). Pharate adults were checked each hour during

[1] Present address: Department of Zoology, University of Washington, Seattle, Washington 98195.

the eclosion gate (Truman, 1971), and emerged animals were separated and held in groups of five or fewer in paper bags during the course of the experiment. They were not fed nor allowed to mate.

Surgical methods

Gland extirpations from pupae were performed following the classical techniques perfected by Williams (1959) on the Cecropia silkmoth pupa, except that no Ringers solution was added to the animal after surgery. Glands were removed to a black watch glass and examined with a dissecting microscope to verify complete removal. A few crystals of 1 : 1 mixture of phenylthiourea and streptomycin sulfate were placed in all wounds. Animals from which the brain had been removed within 24 hours of the pupal ecdysis were injected 10 days later with 8 μg of β-ecdysone dissolved in 50 μl of 10% isopropanol.

Gland extirpations from adult moths were performed approximately 4 hours after adult eclosion. Williams' (1946) technique of continuous CO_2 anesthesia was used, the moth being suspended in the Buchner funnel by placing the neck region in a slit of cardboard which lay across the mouth of the funnel. An incision was made just posterior to the head on the dorsal neck and the CA removed with fine forceps to a black watch glass. Usually, the glands remained attached to a piece of gut or heart. The wound was filled with a piece of Gelfoam (Upjohn Co.).

To remove glands from an adult for subsequent implantation, the head was isolated and all its appendages and the compound eyes removed. Further operations were done with the head submerged in saline. The dorsal surface of the head can then be removed by making two shallow cuts through the cuticle anterior and posterior to the antennal sockets and teasing the cuticle away from the muscles. Removing the remaining musculature and tracheal system exposes the brain and the corpora allata and corpora cardiaca (CC). Appropriate transections with microscissors allows one to remove this entire complex to a black watch glass for observation with a dissecting microscope. The glands which were attached to a piece of heart were removed from the brain. The CA were separated from the CC by one cut. The piece of heart was left attached for easy manipulation of the glands. Implants into adults were made through a lateral incision between the first and second abdominal segment and the wound filled with Gelfoam.

Juvenile hormones

The juvenile hormones or JH mimics were dissolved in light mineral oil (Fisher, Saybolt viscosity 125/135). Fifty microliters of these solutions were injected into the first abdominal segment of the anaesthetized adult using a 26 gauge needle on a disposable 1 ml syringe (Plastipak) controlled by an Agla micrometer. The compounds used in this study were the synthetic Cecropia juvenile hormones, C18-JH (Zoecon Corp., Insect Control) and C17-JH (Insect Control); C16-JH (Insect Control); ethyl 3,7,11-trimethyldodeca-2,4 dienoate (ZR512, Zoecon Corp.); and farnesyl methyl ether (A. M. Ajami).

Dissections

The extent of development of the ovary, in most cases, was determined by the number of mature, fully chorionated eggs present. The abdominal contents of a

moth were removed to absorbent paper and the viscera spread with moderate pressure of the finger. Fully mature eggs resisted pressure whereas immature follicles and fat body were disrupted and absorbed into the paper. The mature eggs could then be counted. Unless otherwise noted, dissections of this type were performed 4 days after adult eclosion.

For observation single ovarioles were dissected out under a modification of a saline developed for *Manduca* (Cherbas, 1973). They were placed in this saline in a black watch glass and observed with a dissecting microscope.

SDS gel electrophoresis

The disc-gel electrophoresis procedures of Davis (1964) were modified and used as an SDS stacking gel system by adding 0.1% SDS (final concentration) to all buffers as suggested by Weber, Pringle, and Osborn (1972). Lower gels were 6% acrylamide and were cast in tubes 5 mm ID. They were 7 cm long. The upper stacking gel was 3% acrylamide, 0.029 M Tris-phosphate, 0.1% SDS and was 200 μl in volume. Samples were added in 50 or 100 μl of sample buffer containing 1% SDS, 0.024 M Tris-phosphate, 10% glycerol by volume, 0.01 M 2-mercaptoethanol, 0.005% bromphenol blue. The electrolyte buffer was 0.01 M Tris, 0.065 M glycine, 0.1% SDS. Electrophoresis was performed at room temperature with a current of 2.5 mAmp per tube for 90 minutes. Gels were stained in 0.01% Coomassie Brilliant Blue (Sigma) for 24 hours and destained for the same length of time. They were scanned with a Gilford 2400 recording spectrophotometer. Percentage of total protein detectable by this method was estimated by weighing cut-outs of the absorbance tracings.

Samples consisted of one μl of hemolymph collected from a cut in the wing of an adult animal as it inflated its wings just after eclosion or by slow centrifugation of older animals with their appendages amputated. Blood was taken from new pupae through cuts in the proboscis and from pharate adults through incisions in the dorsal abdomen. PTU was added to inhibit tyrosinase activity. Blood samples were spun for 2 minutes in a Beckman microfuge. Egg samples were prepared by freezing, thawing, and pipetting mature or immature follicles in a buffer containing 0.024 M Tris-phosphate (pH 6.9) and 1% SDS. The equivalent of one half egg or follicle was added to the gel. All samples were heated in sample buffer at 100° C for 2 minutes.

Results

Normal morphology of ovarian development in the adult

At the time of adult eclosion, a female *Manduca* has no mature eggs in its ovaries. The oldest follicles are 1 mm in length, contain yolk in the oocyte and retain a nurse cell cap (see Figure 1a). During the next 24 hours, these follicles will complete vitellogenesis and the terminal phase of oogenesis (Telfer and Anderson, 1968) including hydration and chorion synthesis (see Figure 1b). The first mature eggs are found in the ovaries 24 hours after eclosion, by 4 days after eclosion, unfed virgin females contain, on the average, 100 mature eggs.

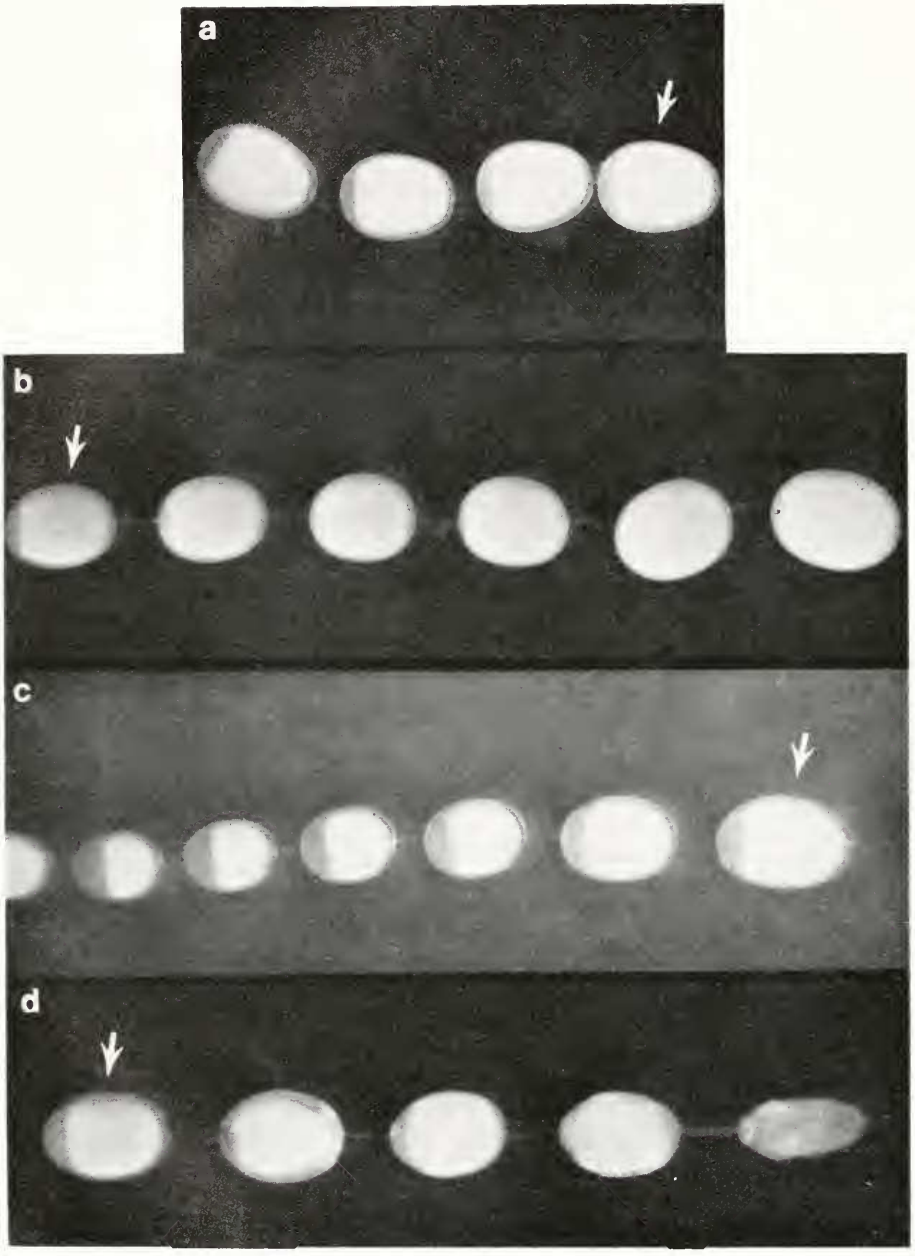

FIGURE 1. Ovarioles of intact and allatectomized females; the anterior end is to the left in all photographs. (a) intact female immediately after eclosion, (b) intact female 12–18 hours after eclosion. Note normal sequence of events beginning with arrow, (c) allatectomized female immediately after eclosion. Follicles appear normal, (d) allatectomized female 48 hours after eclosion. Note abnormal and degenerating follicles immediately posterior to the critical stage (arrow). In all cases arrows indicate follicles at the critical stage.

Effect of allatectomy on egg maturation

Female moths which had been allatectomized as pupae were held for 4 days after eclosion. Upon dissection, no mature eggs were found (Table I) as compared with sham-operated controls which matured almost the normal complement. When the operation was delayed until 4 hours after adult emergence, females were capable of making a small number of eggs in 4 days whereas sham-operated controls produced the normal number.

Although the ovarioles of females allatectomized as pupae appeared normal at the time of eclosion (Figure 1c), they showed widespread degeneration of the posterior follicles at the time of dissection or even within 24 hours after emergence (Figure 1d). In animals deprived of their CA for any length of time as adults, the immature follicles appeared normal until they reached a length of approximately 1 mm, the same stage of development found in the terminal position of the ovariole

TABLE I

Effect of allatectomy on egg maturation in Manduca sexta

Operation	Number animals	Average number mature eggs (± S.E.M.)
No treatment	50	100 ± 6
Allatectomized as pupa	15	0
Sham operated as pupa	10	83 ± 10
Allatectomized as adult	10	7 ± 3
Sham operated as adult	5	97 ± 12

in newly emerged adults. The follicles posterior to this point appeared abnormal with discontinuities in the yolk and disruptions of the nurse cells. Eventually each follicle degenerated.

These results support those of Sroka and Gilbert (1971) who showed that the CA were necessary for egg maturation in *Manduca sexta*. Further, they indicate that the CA do not become active until after adult eclosion. In order to determine the time of onset of activity in the glands, a means of allatectomizing adults was needed which was not so tedious as the surgical procedure.

Newly emerged adults were anesthetized in CO_2 for 1–2 minutes. The animals were decapitated and the wound sealed with melted paraffin. As seen in Table II, this decapitation drastically reduced the ability to mature eggs. The ovarioles contained degenerating follicles, similar to those seen in allatectomized animals.

Removal of the head eliminates three potential endocrine sources: the brain, CC, and CA. To establish the relative importance of these organs, animals decapitated immediately after eclosion received implants of glands 12 hours after emergence. Twelve-hour old females donated either a brain, a CC-CA complex, or an isolated pair of CA. As shown in Table II, only implanted CA restored the ability to form completely mature eggs. (The presence or absence of the CC with the CA made no difference in the response so the results have been combined in the table). Further studies showed that adult male CA were relatively inactive in stimulating egg maturation whereas glands from mid-fifth instar larvae were as active as adult female glands.

TABLE II

Requirement for active CA for egg maturation in decapitated female moths

Treatment after decapitation	Number animals	Average number mature eggs (± S.E.M.)
None	50	1 ± 0.7
Implant adult ♀ brain	14	5 ± 3
Implant adult ♀ CA (1 pr)	20	55 ± 7
Implant adult ♂ CA (1 pr)	5	13 ± 5
Implant larval CA (1 pr)	9	56 ± 17

The implanted CA restored the ability to form only half the normal egg complement in 4 days. Reasons for this will be discussed below.

Dose response curve of JH-induced egg maturation

A series of juvenile hormones and JH mimics were evaluated for their activity in promoting egg maturation in *Manduca*. The animals used for this assay were decapitated within one hour of emergence. The hormone solution was injected 12 hours later and the eggs counted on the fourth day. Only mature, fully chorionated eggs were counted.

Figure 2 shows that the number of eggs matured by decapitated animals depended on the dose of hormone they received. C18-JH and C17-JH were the most active compounds with 1 μg producing three times the control number of eggs in 4 days. C16-JH, a hormone isolated from adult female *Manduca* CA (Judy, *et al.*, 1973), was about 500 times less active, as was the ZR512 mimic. Farnesyl methyl ether, a relatively inactive JH mimic on *Manduca*, was no more

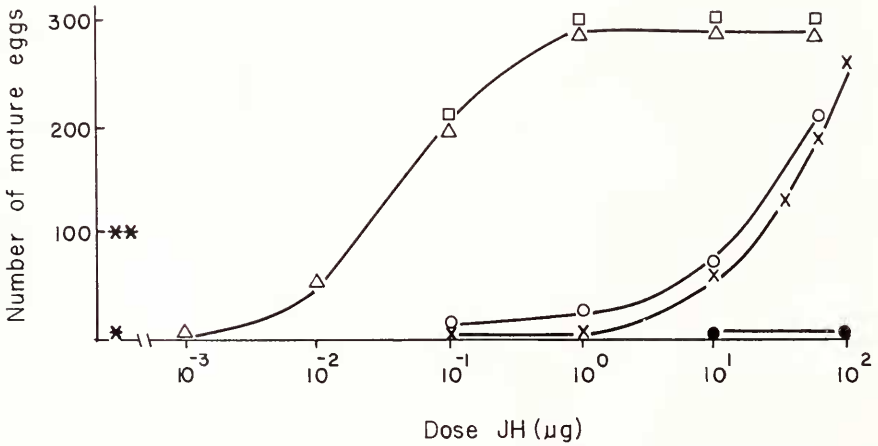

FIGURE 2. Dose-response curve of JH-induced egg maturation. Each point represents the average number of eggs matured in 4 days by 10 animals. Symbols used are: single asterisks, Oil injected controls; double asterisks, untreated females; squares, C17-JH: triangles, C18-JH; open circles, C16-JH; X-ZR512; closed circles, farnesyl methyl ether.

active at 100 μg than pure mineral oil. The order of activity compares favorably with that found in other assays developed on *Manduca* (Truman, Riddiford, and Safranek, 1973; Riddiford and Ajami, 1973).

Effect of brain extirpation on egg maturation

Sroka and Gilbert (1971) reported that removal of the brain from the female pupa resulted in the inability of the subsequent adult to develop eggs, as measured by increase in weight of the ovaries. We removed brains from each of 10 animals within 24 hours of pupation, then injected ecdysone to provoke adult development. All 10 females emerged but none survived for 4 days. Upon dissection they were found to contain no mature eggs, but also showed a curious lack of fat body. In contrast to decapitated animals, these brainless adults were hyperactive. Thus, the subesophageal ganglion without inhibitory influences from the brain seems to cause increased activity in *Manduca*, as Roeder (1935) had previously shown for the mantid, *Mantis religiosa*. This hyperactivity of brainless adults of *Manduca* had apparently caused a quick depletion of their fat body reserves. It was unclear whether they had not developed eggs because of a lack of hormonal stimulus or a lack of nutrient reserves.

To overcome this problem, a second experiment was performed in which the emerging brainless animals were injected with 5 μg of tetrodotoxin (TTX) in 50 μl distilled water. Paralysis by TTX prevented the rapid depletion of the reserves; when the animals were dissected 4 days later, the fat body appeared normal and healthy. None of these animals contained mature eggs (Table III). The morphology of the ovarioles was identical to that of allatectomized or decapitated females in that it showed the same terminal degeneration of follicles. The last normal appearing follicle was 1 mm long and contained yolk and a nurse cell cap. Autopsies showed that at least one CA was present in all animals. TTX-injected controls matured normal numbers of eggs.

Because decapitated animals responded so well to exogenous JH, we thought it unlikely that the brain was providing a second gonadotropic factor. But the possibility remained that the brain secreted a substance before eclosion which was

TABLE III

Effect of brain extirpation and JH injection on egg maturation

Treatment as pupa	Treatment as adult	Number animals	Average number mature eggs (± S.E.M.)
Sham operated	None	10	83 ± 10
Brain removed	None	10	0
Brain removed	5 μg TTX	7	0
None	5 μg TTX	8	125 ± 11
Brain removed	5 μg TTX and 50 μl pure mineral oil	4	0
Brain removed	5 μg TTX and 0.1 μg C17-JH	5	198 ± 16
None	Decapitated and 0.1 μg C17-JH	10	208 ± 20
Brain removed	5 μg TTX and 10 μg ZR512	5	90 ± 9
None	Decapitated and 10 μg ZR512	10	65 ± 7

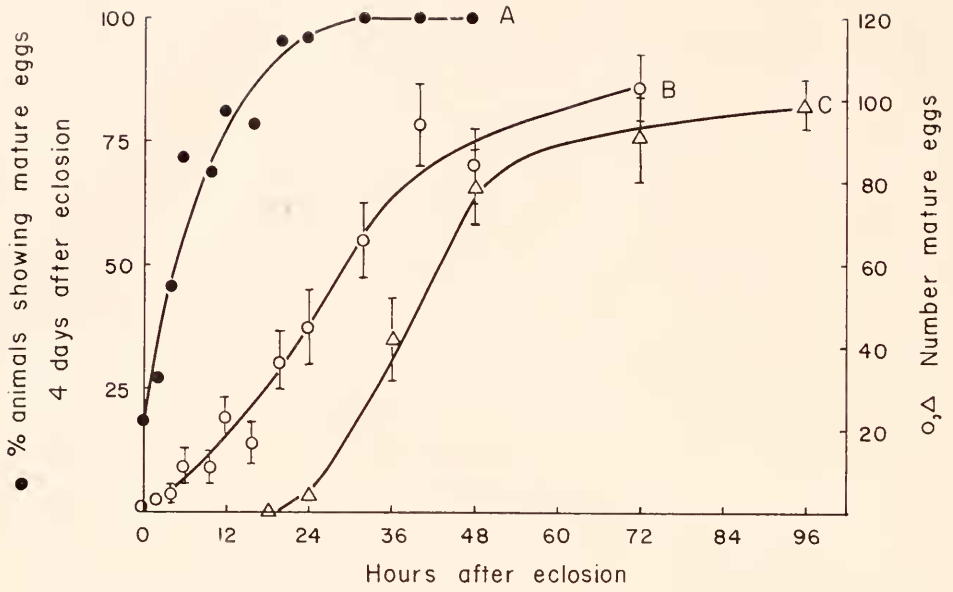

FIGURE 3. Activity of the CA after adult eclosion. Each point represents observations on 51 ± 3 animals except that at 18 hours which represents 20 animals. (A) per cent of animals which have activated CA by time of decapitation at various times after eclosion; (B) number of eggs matured by these decapitated females by 96 hours ± S.E.M.; (C) number of mature eggs present in intact females at various times after eclosion ± S.E.M.

necessary for ovarian development. Therefore, brainless female moths were used as assay subjects for JH. As soon as the brainless animal emerged, it received an injection of 5 μg TTX. Twelve hours later, 50 μl of a JH solution was injected; then the animal was dissected at 96 hours. Controls received 50 μl of pure mineral oil. Table III shows that individuals deprived of their brains throughout adult development responded as well to injected JH as did females decapitated after eclosion.

Activity of the CA in adult females

In order to study the activation of the CA after eclosion and the kinetics of JH release within the population, large numbers of animals were held for various periods of time after emergence and then decapitated. All were dissected on the fourth day after eclosion and the number of mature eggs counted. The results are shown in Figure 3.

Curve A shows that the ability of females to form mature eggs increased when decapitation was performed progressively later in adult life. At the time of emergence, 19% of the population was competent to mature a few eggs (average of 9 eggs) without the continued presence of the CA. Therefore, only a small amount of hormone had been released up to that time since this is only 9% of the normal complement of mature eggs. By 4 hours, 50% of the population had active glands. Decapitation at 32 hours no longer prevented egg maturation in any animal.

The fact that the number of eggs matured was dependent on the amount of JH injected into decapitated females as shown in Figure 2, suggested that the time course of JH release could be estimated by correlating the number of eggs produced in this same set of animals with the time of decapitation. Curve B in Figure 3 represents the rate of JH release. For instance, by 24 hours, enough hormone had been released into the moth's bloodstream to promote the maturation of 45 eggs whereas 12 hours earlier, a moth could mature only 24 eggs when deprived of its source of JH.

Curve C in Figure 3 is a reference curve showing the rate of appearance of mature eggs in intact females. The data for this curve were obtained by dissecting female moths at various times after eclosion and counting the mature eggs present in the ovaries.

The latter two curves show a horizontal displacement of 12 hours at the half-maximum point of 50 eggs. For example, an animal decapitated at 24 hours and dissected at 96 hours contains approximately the number of mature eggs characteristic of a 36 hour old female. This 12 hour period most likely represents the maximum time it takes for a follicle to complete development after becoming

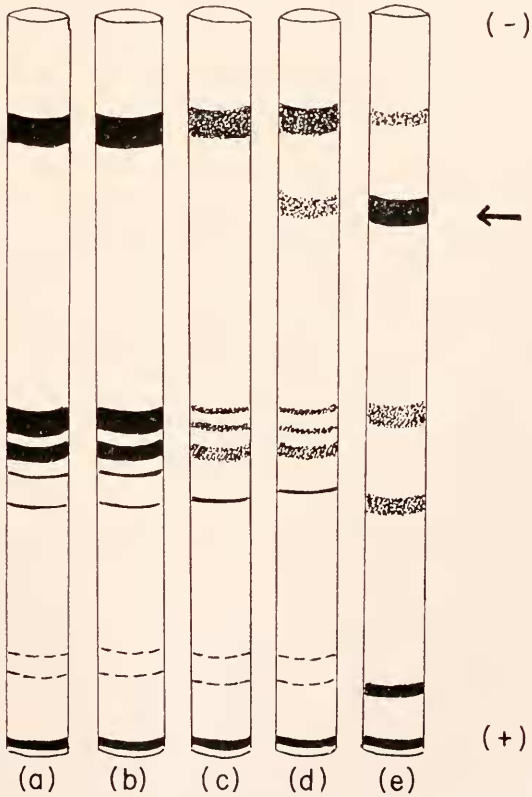

FIGURE 4. Electrophoretograms of hemolymph (a–d) and mature egg homogenate (e): (a) male pupa; (b) female pupa; (c) male adult; (d) female adult; (e) egg homogenate. Arrow indicates vitellogenin.

independent of JH or of the JH-controlled process. It is probable that the period of JH-independence is actually shorter since a finite time is required for the decline of the blood titer of JH.

Effect of allatectomy on vitellogenin concentration

In view of the fact that several insect ovarian maturation systems require JH for synthesis of the female-specific yolk protein, it was of interest to establish the presence of such a vitellogenin in *Manduca* and to determine the effect of allatectomy on its concentration in the blood and uptake into follicles.

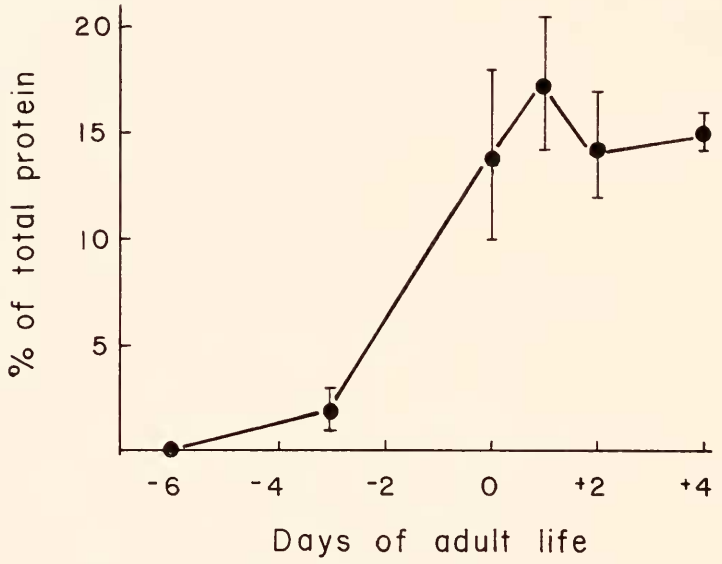

FIGURE 5. Concentration of vitellogenin in blood during development. Each point represents the mean value of 5 samples. Error bars show standard deviation.

When blood samples of early pupae and freshly-emerged adults were subjected to SDS gel electrophoresis, a sex-specific protein band was found in the adult female (see Figure 4). The same band was found to be a major protein component of the mature egg. This is, presumably, the vitellogenin protein.

In order to determine the time in development when the vitellogenin first appeared in the blood, hemolymph samples were withdrawn from developing adults at 3 day intervals after pupal ecdysis. No vitellogenin was found in animals immediately after pupation nor during early adult development. In the 18 day period between pupal ecdysis and adult eclosion, vitellogenin was first detected in the blood with this technique 3 days before eclosion. During adult life, as shown in Figure 5, the yolk protein comprised approximately 15% of total blood protein which stained with Coomassie Brilliant Blue. This stain is quite sensitive and will detect less than 0.5 μg of protein in a single band (Weber, *et al.*, 1972).

Figure 6 shows the densitometer tracings from gels of female blood taken just after eclosion from an intact female and from one which had been allatectomized as an early pupa. The vitellogenin band was present in a similar concentration in both. Figure 7 shows the densitometer tracings from gels of homogenates of

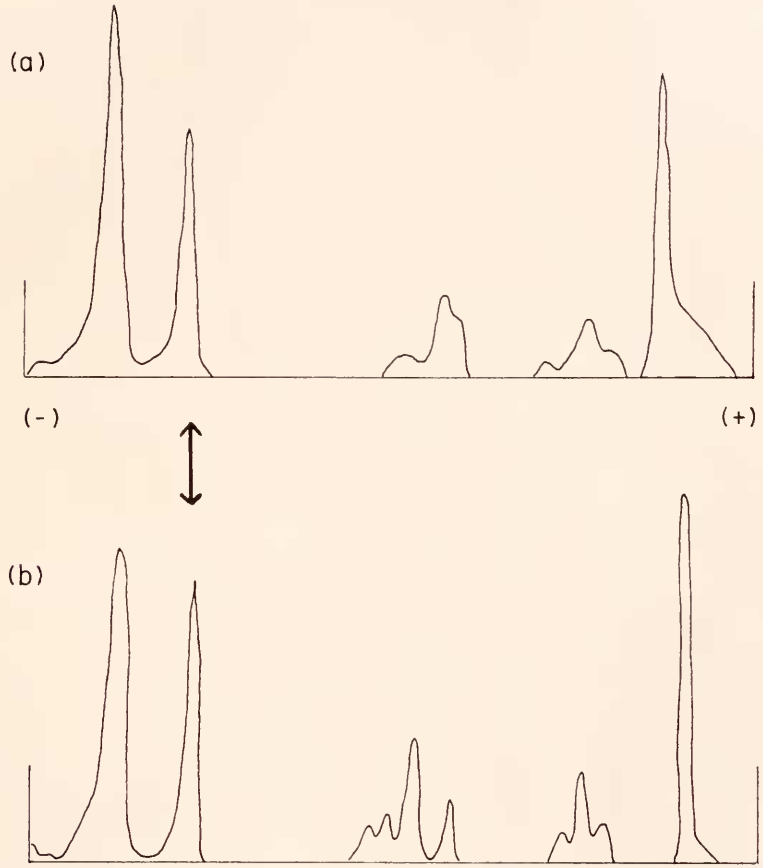

FIGURE 6. Densitometer tracings of gels of hemolymph from (a) intact female and (b) female allatectomized as pupa. Blood samples were taken immediately after eclosion. Arrow indicates vitellogenin.

immature follicles (1 mm long) of intact or of allatectomized females at the time of eclosion. There were no major differences between the two, indicating that vitellogenesis proceeded normally, at least up to this critical stage of development.

DISCUSSION

The results of the experiments reported here confirm that adult *Manduca sexta* females require JH for complete egg maturation. The CA, which cease secretion in the fifth instar larva (Nijhout and Williams, 1974) and remain quiescent during adult development, become active at about the time of eclosion in female

moths. This activation of the CA in adults appears to be controlled by the brain as brainless animals do not mature eggs. Yet they will respond to appropriate doses of exogenous JH by making twice the number of eggs found in intact

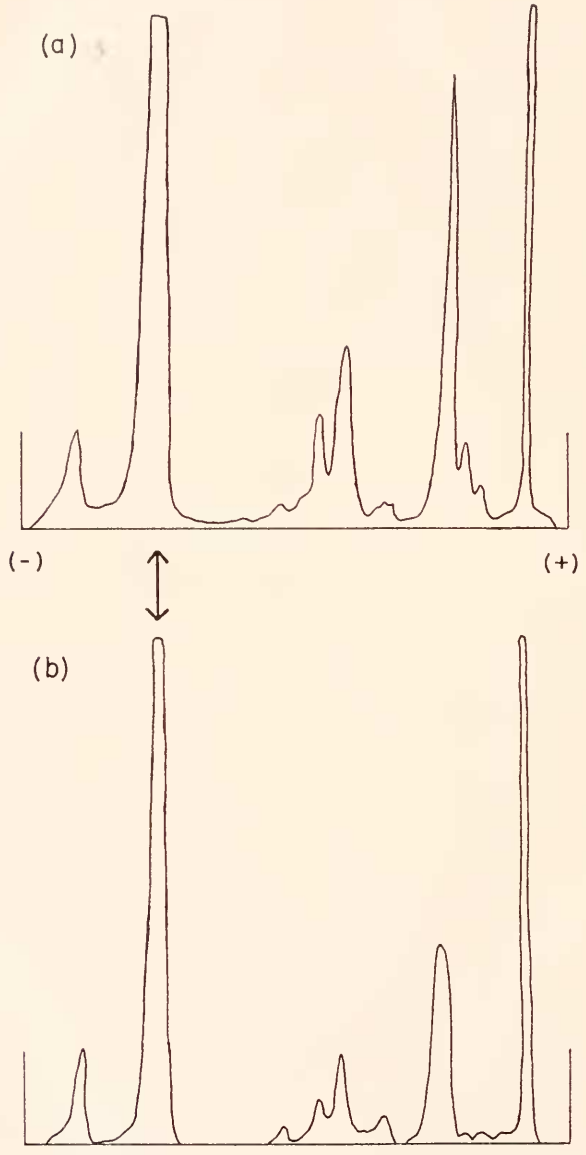

FIGURE 7. Densitometer tracings of homogenates of immature follicles, 1 mm long from (a) intact female and (b) female allatectomized as pupa. Follicles were dissected at the time of eclosion. Arrow indicates vitellogenin.

females. Therefore, the brain somehow activates the CA but plays no further role in ovarian development.

The egg maturation assay indicates that the various juvenile hormone compounds produced by adult female *Manduca* CA do not have similar activities. Judy *et al.* (1973) found that intact glands, *in vitro*, secreted C-16 and C-17 JH into the medium. Reibstein and Law (1973) have reported that homogenates of adult female glands produced C-18 JH as well. Our results, along with those of Truman *et al.* (1973) and Riddiford and Ajami (1973) would support C-17 and/or C-18 as the active hormone(s) in this species.

Once activated, the CA continue to release JH for about two days in virgin, unfed females. Apparently, about this time, the glands shut off since the rate of egg maturation in an intact female decreases from 3.2 to 0.5 eggs/hour at 48 hours after eclosion (Figure 3C). Similarly, females decapitated before the end of the second day do not form the maximum number of eggs, whereas those decapitated on the third day or later mature the normal complement (Figure 3B). The decreased rate of egg maturation in intact females cannot be due to a depletion of nutritional reserves as Figure 2 shows that unfed females are capable of making up to 300 eggs in 4 days when given an appropriate dose of JH. The JH was injected in a light mineral oil vehicle so as to allow a slow continuous release of hormone into the blood (Riddiford and Ajami, 1973); thus, with the optimum doses, approximately 3.0 eggs/hour were matured during the entire period following injection.

The mechanism which results in the relative inactivity of the CA after the second day of virgin life is unknown. We suspect that the virgin condition results in the inactivation of glands and that some sort of "mating stimulus" would maintain their activity or reactivate them. Mating stimuli, both mechanical and humoral, which stimulate egg production and/or oviposition are known for various insects (see Engelmann, 1970; deWilde and deLoof, 1973; Riddiford and Ashenhurst, 1973). Often these stimuli act via the brain. In view of the fact that the brain activates the CA in *Manduca*, it may also provide a constant stimulation for them. The necessity for the brain in the maintenance of secretion by the CA would explain why glands implanted into decapitated animals sustain the maturation of only half the normal number of eggs. Reimplantation of CA into animals allatectomized as pupae (which therefore have intact brains) restored normal ovarian development as measured by the increase in weight of the gonad (Sroka and Gilbert, 1971).

The role of JH in adult female *Manduca* is novel when compared to its effects in adults of other species that have been studied. The hormone is not necessary for synthesis and accumulation of vitellogenin in the blood nor for its incorporation into the oocyte of less than 1 mm in length. Yet follicles do not complete development in its absence. A critical period can be defined for each follicle. Independent of JH, a follicle can reach that stage characteristic of the terminal follicles of a newly emerged moth. The follicle is 1 mm long, contains a relatively large amount of yolk in the oocyte, but retains a nurse cell cap. Normal development beyond this critical stage requires JH. In its absence, abnormalities appear in both the nurse cells and oocyte. Eventually, all the contents of the oocyte are resorbed and the follicle shrivels. The critical stage is not a resting stage which lies

quiescent until exposed to the hormone. As each follicle reaches and passes the critical period, it degenerates.

Similarly in *Aedes aegypti* (Gwadz and Spielman, 1973), the brain activates the CA soon after emergence in the adult female. JH then acts, perhaps directly on the immature follicles, to allow their development to the normal resting stage. From this point, stimuli initiated by the blood meal are needed to begin vitellogenesis. The temporal sequence in *Manduca* female moths is the same except that at the time of eclosion, terminal follicles have already completed most of vitellogenesis. JH acts at this point to allow normal development beyond the critical stage.

Our results are consistent with the hypothesis that JH either acts directly on the developing follicle or that it drives an extra-ovarian process which maintains the correct environment for follicular development. It is obvious that JH is not a trigger for an event or series of events, which, once initiated, no longer require the presence of the hormone to allow the female to mature 100 eggs. If this were true, Curve B in Figure 3 would rise more sharply with respect to Curve C. The fact that the curves are parallel over most of their length indicates that the release of hormone itself is the rate limiting step. We do not know the length of the JH-dependent stage for each follicle; but once the follicle becomes independent of the hormone, it requires a maxium of 12 more hours to reach maturity.

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Summary

1. In *Manduca sexta* females, follicles develop independently of the corpora allata until they reach a critical stage in late vitellogenesis. In the absence of juvenile hormone (JH), follicles then degenerate. When JH is present, the follicles continue development; they then become independent of JH for the final 12 hours of egg maturation.

2. Allatectomy of the pupa of *Manduca sexta* or the newly eclosed adult female prevents egg maturation as does extirpation of the brain from the pupa or decapitation of the adult. The ability to develop follicles beyond the vitellogenic phase is restored by injection of synthetic JH or JH mimics.

3. The brain activates the corpora allata close to the time of adult emergence. The glands secrete JH at a constant rate for 48 hours and then become less active in virgin, unfed moths.

4. A female specific protein, identified by SDS gel electrophoresis, is present in adult blood and egg homogenates. Allatectomy has no effect on the concentration of vitellogenin in the blood nor on its incorporation into immature follicles less than one mm long.

5. The action of JH in promoting egg maturation in *Manduca sexta* appears to be different from that in most other well-studied insect systems in that the hormone is not required for the synthesis and/or uptake of the female-specific yolk protein. Rather it is required for a critical step in development.

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